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SHORT COMMUNICATION

Extraintestinal helminths of the common vole (*Microtus arvalis*) and the water vole (*Arvicola terrestris*) in Western Austria (Vorarlberg)

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Abstract Between September and December 2004, a total of 411 voles (318 common voles and 93 water voles) were caught in the Austrian province of Vorarlberg (Lustenau, Hohenems, and Dornbirn) and were examined by macroscopy, microscopy, and molecular biological analysis to determine the presence and extent of medically important extraintestinal helminths. The following extraintestinal helminth species were detected: *Taenia taeniaeformis* (liver), *Calodium hepaticum* (liver), and *Echinococcus multilocularis* DNA (liver) in the common vole; and *Taenia taeniaeformis* (liver), *Calodium hepaticum* (liver), and *Taenia crassiceps* (musculature) in the water vole. Infestations with *Toxocara canis* and *Trichinella* sp. were not found. Our study documents the first description of *E. multilocularis* DNA in the intermediate host (*Microtus arvalis*) and of other medically relevant extraintestinal helminths in common and water voles in Austria.

Introduction

Voles are known as hosts of different helminth species which are directly (i.e., *Calodium hepaticum*) or indirectly (i.e., *Echinococcus multilocularis*) transmittable to humans. Some of them have the capability to cause severe diseases in the human host (e.g., hepatic capillariosis, alveolar echinococcosis), others are rarely detected in humans (i.e., *Taenia taeniaeformis*, *Taenia crassiceps*, *C. hepaticum* (Sterba and Barus 1976; Arocker-Mettinger et al. 1992; Ekanayake et al. 1999; Juncker-Voss et al. 2000)). However, among these are helminths known as important pathogens particularly in immunodeficient patients (Heldwein et al. 2006; Francois et al. 1998; Auer et al. 2000).

Despite the medical relevance of vole-borne helminths, the knowledge of the extraintestinal helminth fauna of murides in Austria is rather limited. From former epidemiological studies we know that the following helminths occur in Austrian rodents: *T. crassiceps* in the snow vole (*Chionomys nivalis*) and the muskrat (*Ondatra zibethicus*) (Pfaller and Tenora 1972), *T. taeniaeformis* in the snow vole (Pfaller and Tenora 1972), and *C. hepaticum* in the field vole (*Microtus agrestis*), common vole (*Microtus arvalis*), house mouse (*Mus musculus*), long-tailed field mouse (*Apodemus sylvaticus*), and the Norway rat (*Rattus norvegicus*; Frank 1977; Juncker et al. 1998). Attempts to detect *E. multilocularis* metacestodes in voles and other murides have failed so far (Pampas 1994). Studies on the prevalence of *Toxocara* sp. and *Trichinella* sp. in voles have never been performed in Austria.

Due to the fact that the most western province of Austria, Vorarlberg, is known as an endemic area of *E. multilocularis* in foxes with prevalences up to 43.8% in the district of Feldkirch (Prosl and Schmid 1991), the aim of the current study was to prove the occurrence of metacestodes of *E. multilocularis* on one hand and other medically relevant, extraintestinal helminths in voles in Western Austria on the other hand. Macroscopy, microscopy, and molecular biological methods were used to examine the brain, liver, and musculature of common and water voles for *T. taeniaeformis*, *T. crassiceps*, *E. multilocularis*, *C. hepaticum*, *Toxocara canis* and *Trichinella* sp.

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Material and methods

Between September and December 2004, a professional pest controller caught a total of 411 voles (318 common voles and 93 water voles) in three districts (Hohenems, Lustenau, Dornbirn) of the most western Austrian province, Vorarlberg. The voles were stored at –18°C and were brought to our institute. In order to exclude mix-ups with field voles (*M. agrestis*), a species classification of common voles was conducted during necroscopy with an inspection at the inner side of the second molars of the upper jaw.

During necroscopy, the pleural and peritonal cavity and the skull were opened. The inner organs and the musculature were examined macroscopically for abnormalities. Any metacestodes found were transferred into tubes with 70% ethanol. From each vole the liver, brain, diaphragma, and the Musculus masseter were removed for further examinations. If abnormalities were found on the surface of the liver (e.g., white spots) a sample of those livers was taken aside to undergo molecular biological analysis for *E. multilocularis* DNA ($n_{\text{common voles}} = 102$; $n_{\text{water voles}} = 40$). Furthermore the remnants of abnormal liver parts were isolated for the microscopical examination for *C. hepaticum* eggs and larval cestode hooks. Therefore, inconspicuous liver parts were cut into small slices and homogenized with a sodium chloride solution through two tissue sieves with a mesh size of 380 and 46 µm. The sieve was washed out, and the cell solution was controlled microscopically for the presence of hooks of larval cestodes and *C. hepaticum* eggs. Macroscopically healthy liver parts were also cut into small slices, subjected to an artificial digestion method (Cheesbrough 1998) and examined microscopically for the presence of *C. hepaticum* eggs and metacestode hooks.

The larval cestodes were counted and their scoleces were prepared with Berlese's fluid (Reichenow 1969) for species classification (Schmidt 2001).

The removed diaphragma and Musculus masseter were examined for the presence of *Trichinella* sp. microscopically.

Molecular biological methods (PCR) with specific primers were used to detect DNA of *E. multilocularis* (liver) (Schneider et al. 2008), *Taenia* sp. (metacestodes) (Kocher et al. 1989; Rodriguez-Hidalgo et al. 2002), and *T. canis* (brain; Bowles et al. 1992; Jacobs et al. 1997).

Results and discussion

In total, *T. taeniaeformis* cysts were detected in 22 common and in 30 water voles, *T. crassiceps* metacestodes in the musculature of two out of 98 water voles and *E. multilocularis* specific DNA fragments in one out of 102 common voles (Table 1). Furthermore 34 isolated metacestodes were confirmed using *Taenia*-specific PCR techniques. In addition *C. hepaticum* (eggs and one adult) was detected in three common and in one water vole. Morphological examinations for *Trichinella* sp. and molecular examinations for *T. canis* DNA revealed negative results.

Voles are natural hosts of several parasitic helminths which are transmitted to humans by oral ingestion of infective eggs excreted by the final hosts, i.e., foxes, cats, and dogs (*E. multilocularis*, *T. taeniaeformis*, *T. crassiceps*, *Toxocara canis)*, as well as infective eggs released from the liver of dead voles (*C. hepaticum)* or *Trichinella* larvae in undercooked meat of wild boars feeding infected voles. In our study the most frequent extraintestinal helminth was *T. taeniaeformis*, which could be detected in 22 common voles (6.9%) and 30 water voles (32.3%). Up to two metacestodes (in *M. arvalis*) and up to 15 metacestodes (in *Arvicola terrestris*) could be observed in the livers of the examined voles, respectively, which could also be observed by Murai (1982). This is the first account of *T. taeniaeformis* in common and water voles in Austria.

Thirty-one and 43 larval stages of *T. crassiceps* were found in the neck musculature of two water voles (2.2%; first finding in Austria), which is slightly higher than observed in

Table 1 Overview on the prevalences of human pathogenic extraintestinal helminths in common (*Microtus arvalis*) and water voles (*Arvicola terrestris*) in the Austrian province Vorarlberg

Helminth species	Vole species	Source	Prevalence[a]
Echinococcus multilocularis	Common vole	Liver	1 (0.98)[c]
Taenia taeniaeformis	Common vole	Liver	22 (6.9)[b]
	Water vole	Liver	30 (32.3)[b]
Taenia crassiceps	Water vole	Musculature	2 (2.2)[b]
Calodium hepaticum	Common vole	Liver	3 (0.9)[b]
	Water vole	Liver	1 (1.1)[b]

[a] Number (percent) of voles infected

[b] Based on examination (macroscopy, microscopy, and molecular biology) of 318 common voles and 98 water voles

[c] Based on molecular biological analysis (PCR) of livers of 102 common voles and 40 water voles

France (0.1%; Deblock and Petavy 1983; Petavy et al. 1985) and in Germany (0.22%; Schaerer 1987).

C. hepaticum (eggs and parts of an adult worm) were found in three common (0.9%) and in one water vole (1.1%; first finding in Austria). Thirty years ago, Frank observed *C. hepaticum* in four common voles in the Neusiedlersee region in Eastern Austria (Frank 1977). In Switzerland too, a low prevalence (0.2%) in water voles has been observed (Reperant and Deplazes 2005) whereas in Russia infection rates of up to 20.6% have been documented in *M. arvalis* (Romašov 1983).

Reports of double infections with two different helminth species in voles are rare (Petavy et al. 2003); in our study two co-infections were found: *T. taeniaeformis* and *T. crassiceps* in one water vole and *T. taeniaeformis* and *C. hepaticum* in one common vole.

Although the Austrian province Vorarlberg has been known as an endemic area of *E. multilocularis* in foxes (Prosl and Schmid 1991, Duscher et al. 2006) and of human alveolar echinococcosis (Auer and Aspöck 1991), *E. multilocularis* could not be detected in Austrian rodents so far. Within our study, it was not possible to find metacestodes of *E. multilocularis*. However, we have—at least—been able to find specific DNA in the liver of one common vole and are able to close and verify the sylvatic life cycle of *E. multilocularis* in Vorarlberg. On the other hand, our efforts to detect *Trichinella* larvae or *Toxocara* DNA in *M. arvalis* or *A. terrestris* in Vorarlberg proved to be unsuccessful.

The results of our study can be summarized as follows:

1) *T. taeniaeformis* could be detected in *M. arvalis* and *A. terrestris* in Austria for the first time.
2) *T. crassiceps* and *C. hepaticum* could be detected in *A. terrestris* in Austria for the first time.
3) *E. multilocularis* DNA could be detected in *M. arvalis* in Austria for the first time.
4) Neither *Trichinella* sp. larvae nor *T. canis* DNA could be found in common or water voles.
5) Thus, this study represents not only a contribution to the knowledge of the helminthic fauna of voles in Western Austria, but it also documents the autochthonous existence of parasites with human-medical relevance for immunocompetent patients (i.e., *E. multilocularis*) and particularly for persons with immunodeficiency (i.e. *T. crassiceps*) in Vorarlberg.

Ethical standards All voles were caught in 2004 according to the Vorarlberger law Lg Bl. Nr. 50/2002.

Conflict of interest The authors declare that they have no conflict of interest.

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